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# Surface Modification of Nanoparticles Used in Biomedical Applications

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## 1. Introduction

In the last two decades a lot of attention has been paid on the preparation of nanoscaled materials and recently, depending on the development of new fabrication and characterization techniques, materials composed of a few atoms up to hundreds of atoms can be synthesized and their properties determined easily. Nano sized materials, as compared to their bulk counterparts, exhibit new characteristic optical, electrical and magnetic properties due to the enhanced surface to volume ratio and quantum confinement effects emerging in these size ranges. These new features of nanoparticles offers them the possibility to be used in a wide range of technological (magnetic data storage, refrigeration), environmental (catalysts, hydrogen storage), energy (lithium-ion batteries, solar cells) and biomedical applications. In biomedical applications there are different kind of nanoparticles used like metallic [1], magnetic, fluorescent (quantum dot) [2,3], polymeric [4,5] and protein-based nanoparticles [6, 7], in which much of the research in this field is focused on the magnetic nanoparticles. In this review only magnetic nanoparticles, which are composed of a magnetic core surrounded by a functionalized biocompatible surface shell will be concerned, where several reviews on other type of nanoparticles are available in the literature. In the scope of the text particular attention will be paid on superparamagnetic iron oxide (SPIONs), which is beyond the most studied one among all types of magnetic nanoparticles. In the beginning of the article, the biomedical applications of magnetic nanoparticles are summarized together with the key factors effecting the nanoparticles' performance in these applications. Then the requirement for the surface treatment of the nanoparticles are discussed in the context of colloidal stability, toxicity (biocompatibility) and functionalization. Finally, the followed procedures for the surface coating of magnetic nanoparticles are briefly explained and the different materials used as surface coatings are listed in detail with examples from literature.

## 2. Biomedical applications of magnetic nanoparticles

Magnetic nanoparticles (MNP) with dimensions ranging from a few nanometers up to tens of nanometers, thanks to their comparable or smaller size than proteins, cells or viruses, are able to interact with (bind to or penetrate into) biological entities of interest [8]. These size advantages of MNPs together with their sensing, moving and heating capabilities based on the unique nanometer-scale magnetic and physiological properties give them the possibility to be used in biomedical applications such as magnetic resonance imaging (MRI), targeted drug delivery and hyperthermia [9].

In MRI, which is the most promising non-invasive technique for the diagnosis of diseases, MNPs are used as contrast enhancement agents [10-12]. The improved contrast in MR images permits better definition and precise locating of diseased tissues (such as tumors) together with monitoring the effect of applied therapy. The operation of MRI is based on the Nuclear Magnetic Resonance (NMR) phenomena and the image processing is realized by spatially encoding of NMR signal of water protons which comes from different volume elements in the body called voxels. The image contrast in MRI depends mainly on proton density, spin-lattice ( $T_1$ ) and spin-spin ( $T_2$ ) nuclear relaxation times, differently weighted along different parts (voxels) of the body. The so-called contrast agents (CAs) themselves do not generate any signals, yet they contribute to the nuclear relaxation of water protons by creating local magnetic fields, which are fluctuating in time through different mechanisms like magnetization reversal and water diffusion [13]. As a consequence, the CAs decrease or increase the MRI signal intensity in the tissues by shortening both the  $T_1$  and  $T_2$  relaxation times of nearby protons resulting darker or brighter points in the image. The contrast enhancement efficiency of CAs is measured by the relaxivity  $r_{1,2}$ , which is defined as the increment of the nuclear relaxation rates  $1/T_{1,2}$  of water protons induced by one mM of the magnetic ion. The CAs having a ratio  $r_2/r_1$  greater than two, especially at magnetic fields mostly used in MRI tomography (0.5, 1.5 or 3 Tesla), are classified as  $T_2$ -relaxing (or negative) CAs since they more effectively decrease  $T_2$  rather than  $T_1$ . On the other side CAs, characterized with a ratio  $r_2/r_1$  smaller than two, have more pronounced effect on  $T_1$  and hence called as  $T_1$ -relaxing (or positive) contrast agents [14]. The MNPs showing superparamagnetic property at physiological temperatures generally serve as  $T_2$ -relaxing CAs and they negatively improve the image contrast resulting darker spots where they are delivered. Commercially a wide variety of superparamagnetic iron oxide (SPIO) based negative CAs are available in the market like Endorem, Sinerem, Resovist, Supravist, Clariscan, Abdoscan etc., where each of them are used for different purposes or in different organs in clinical MRI application.

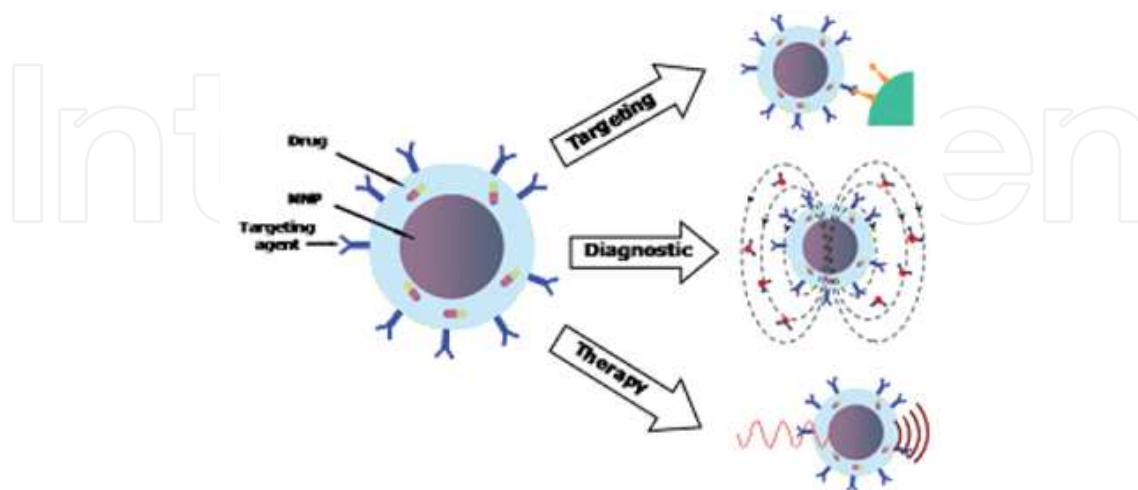
In a second biomedical application called magnetic hyperthermia, which is a thermally treatment of cancerous cells based on the fact that the cancer cells are more susceptible to high temperatures than the healthy ones, the MNPs can be used as heating mediators [15,16]. In this technique after concentrating the MNPs in the region of malignant tissue (by targeting or by direct injection), the MNPs are made to resonantly respond to a time-varying magnetic field and transfer energy from the exciting field to the surroundings as heat. By this way using an alternating field with sufficient intensity and optimum frequency, the temperature of tissue

can be increased above 40-42 °C and the infected cells could be selectively destroyed. According to the models describing the heat release mechanisms of MNPs, increasing the frequency and the amplitude of the alternating field promises to significantly enhance the amount of released heat, but the limitations imposed by the biological systems restrict these values under a few tens of kA/m and a few hundreds of kHz for the field strength and frequency, respectively [17]. The MNPs' heating capacity in magnetic hyperthermia is denoted by Specific Absorbtion Rate (SAR) or in another term Specific Loss of Power (SLP), which is a measure of the energy converted into heat per unit mass. As the similar case in MRI CAs, the majority of magnetic hyperthermia heat mediators investigated to date are based on iron oxide MNPs, where in these studies the typical values reported for the maximum attained SAR range between 10 and 200 W/g [18-20]. However, it has been also focused on several systems alternative to iron oxide, where in some of them SAR values 3-5 times larger than those of similar iron oxide MNPs are attained for the same field parameters [21-22]. Recently, although there are lots of in-vitro studies about magnetic hyperthermia, the therapy with hyperthermia is still in pre-clinical stage and only a few studies on human patients are reported [23,24]. However, in cancer treatment the magnetic hyperthermia is thought to be introduced as a complementary technique to chemo- and radiotherapy as increasing the effects of these therapies [25, 26].

In another major in-vivo application, the MNPs are used as drug carriers in a magnetic 'tag-drag-release' process called targeted drug delivery. In a drug delivery process the MNPs, loaded with special drug molecules or conventional chemotherapy agents, are directly vectorized to tumor cells by targeting ligands on their surfaces or they brought into the vicinity of target tissue through magnetic forces exerted on them under an applied external magnetic field. Once the drugs/carriers are concentrated at the diseased site, the drugs are released from the carriers, again through modulation of magnetic field, enzymatic activity or changes in physiological conditions such as pH, osmolality or temperature. With this approach, the tumor cells can be destroyed by concentrating only the required quantity (dose) of drugs at target specific locations with minimized side effects on healthy tissues. The performance of the application depends mainly on the drug release kinetics and the cellular uptake of MNPs in tissues. There are huge number of drug delivery studies in the literature reporting both in-vitro and in-vivo results on different cell cultures and different types of tumors respectively, where in these studies several kind of targeting ligands and anticancer drugs are tested and in most of them again superparamagnetic iron oxide is used as magnetic core [27,28]. Generally in the design of MNPs for targeted drug delivery, in order to monitor the effect of the therapy, MRI contrast increament ability of the same MNP system is also investigated [29].

Actually in recent years much more interest has been concentrated on multifunctional MNPs, in which the above mentioned diagnostic (MRI) and therapeutic (hyperthermia and drug delivery) capabilities are combined [15,30,31]. Although some MNP-based MRI contrast agents are commercially available on one side and magnetic hyperthermia is already utilized in conjunction with other kind of therapies on the other side, MNPs optimized to perform both functions (diagnostic and therapeutical) have not been developed yet. Indeed the possibility to associate therapeutic effect generated by the heat release and delivered drugs with the enhanced contrast in MRI images, is extremely appealing since it would provide the

possibility before heating the tissue to track the particle distribution by MRI, and after the drug therapy or thermotherapy to have an immediate control of the efficacy of the treatment itself. In Figure.1 a summarized illustration of biomedical applications has been shown.



**Figure 1.** Biomedical applications of magnetic nanoparticles (image has been reproduced from A. Lasicalfari et al. [9].

### 3. Design of MNPs for biomedical applications

In many biomedical applications of MNPs, usually a core/shell structure is required, where the inorganic magnetic core is surrounded by an outer layer of shell (coating). The successful design of MNPs needs a careful selection of magnetic core and surface coating material, where the first mainly determines the MNPs' above mentioned heating, sensing etc. abilities related with application efficiency and the second specifies the interaction of these MNPs with physiological environment. In following sections the properties of magnetic core and the surface coating are discussed in detail.

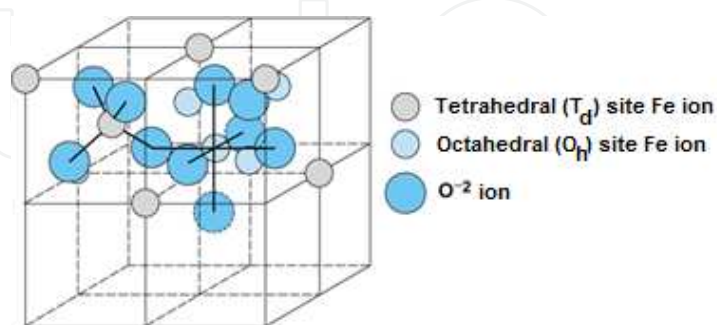
#### 3.1. Magnetic core

In the selection of magnetic core some important aspects should be taken in the consideration, such that for the first the magnetic core should be crystalline and smaller than a critical size as to consist of only one magnetic domain. This ensures that the single-domain nanoparticles exhibit superparamagnetic behaviour with zero remanent magnetization in the absence and a very high magnetization (approximately three orders of magnitude greater than paramagnetic materials) in the presence of an external field. This physical phenomenon is the key requirement for biomedical applications since the particles can be dispersed and concentrated in solution in-vitro or in blood circulation in-vivo, without forming magnetized clusters and they can also respond to an instantly applied field with some kind of magnetic on/off switching behaviour. For the second aspect, the size distribution of the magnetic cores should be as narrow as possible and third, all the magnetic cores in a particular sample should have a unique



and uniform shape (monodispersed). This is because all the magnetic and physico-chemical properties strongly depend on the size and shape of the magnetic cores. From an applicative point of view the size, properly speaking the hydrodynamic size which is the total diameter of MNP including the coating thickness, is also important for the elongation of MNPs' circulation time in blood and for the improvement of their internalization by the cells at the target tissue such that smaller nanoparticles have bigger chance to reach the target cells and to penetrate inside them.

In the search of suitable elements for the magnetic core of MNPs, among other magnetic materials transition metals like Fe, Ni, Co and Mn are good candidates since they offer high magnetization values which is important for high performance MRI and hyperthermia applications. However they are not stable and oxidate very quickly yet in the synthesis step, if they are not specially treated. For this reason transition metal oxide compounds (also called ferrites) which are stable and have acceptable magnetizations are generally introduced in biomedical applications. Superparamagnetic iron oxide (SPION), belonging to ferrite family, is the most commonly employed one in biomedical applications. Nanocrystalline iron oxides have an inverse spinel crystal structures, where the oxygen atoms form face centered cubic lattices and iron ions occupy tetrahedral ( $T_d$ ) and octahedral ( $O_h$ ) interstitial sites (Figure.2). Iron oxide generally exist as two stable forms called magnetite ( $Fe_3O_4$ ) and its  $\gamma$  phase maghemite ( $\gamma-Fe_2O_3$ ), but there is also a  $\alpha$  phase called hematite ( $\alpha-Fe_2O_3$ ), which is not stable and obtained by thermal treatment of magnetite or maghemite. In magnetite, bivalent  $Fe^{+2}$  ions occupy  $O_h$  sites and trivalent  $Fe^{+3}$  ions are equally distributed between  $O_h$  and  $T_d$  sites, whereas maghemite, which can be result from the oxidation of magnetite, only contains  $Fe^{+2}$  ions distributed randomly over  $O_h$  and  $T_d$  sites [32]. In magnetite, since there is the same number of  $Fe^{+3}$  ions in  $O_h$  and  $T_d$  sites, which compensate for each other, the resulting magnetization arises only from the uncompensated  $Fe^{+2}$  ions in  $O_h$  sites. On the other side the magnetization of maghemite originates from uncompensated  $Fe^{+3}$  ions. However their magnetic behaviours and other properties are quite similar, which makes it very difficult to distinguish between them.



**Figure 2.** The cubic inverse spinel crystal structure of iron oxide showing  $T_d$  and  $O_h$  sites

Alternatively other types of ferrites were also studied for biomedical applications. In these ferrites, as compared to the iron oxide nanocrystals,  $Fe^{+2}$  ions are fully or partially replaced by other transition metals in spinel structure and they represented by a general formula

( $\text{MFe}_2\text{O}_4$ ;  $\text{M}=\text{Zn, Ni, Co, Mn}$ ). Manganese ferrites ( $\text{MnFe}_2\text{O}_4$ ) serve as potential MRI contrast agents with largest magnetization among other ferrites [33] and zinc ferrite ( $\text{ZnFe}_2\text{O}_4$ ) nanocrystals demonstrated better MRI contrast with respect to similar magnetite nanocrystals [34]. On the other side as regard to magnetic hyperthermia, the use of cobalt ferrites ( $\text{CoFe}_2\text{O}_4$ ), known by its high magnetic anisotropy energy which is responsible for holding the magnetization along certain direction, has proven to be a good way since much higher heating rates were reported for these nanocrystals compared to other ferrites [21]. Another strategy followed in the design of magnetic core for biomedical applications is the synthesis of mixed ferrites, where simple ferrites including one kind of magnetic ion except iron are doped with other kind of magnetic ion. This is generally realized in order to utilize from different outstanding magnetic features of different ions. For example in hyperthermia application, Co, being a hard magnetic material, is doped to other ferrites ( $\text{MFe}_2\text{O}_4$ ) in changing concentrations ( $\text{Co}_x\text{M}_{1-x}\text{Fe}_2\text{O}_4$ ;  $x=\text{concentration}$ ) in order to increase the magnetic anisotropy eventually to improve the heat transfer rate, whereas Zn is added for reducing the Curie temperature of resulting mixed ferrite [35]. This latter operation permits the tuning of the maximum reached temperature by heat transfer and prohibits overheating of healthy tissues via the process called self-controlled hyperthermia [36]. Table.1 summarizes some important magnetic parameters of transition metal oxides (ferrites) used in biomedical applications.

Another class of materials used as magnetic cores are the magnetic alloy nanoparticles, which composed of two or three different kind of metals like FeCo, FePt and NiCu. FePt is the most famous one among these materials due to its chemical stability and high magnetic anisotropy. Maenosono and Saita have studied FePt nanocrystals and proposed them to be used as high performance contrast agents and heating mediators in MRI and magnetic hyperthermia, respectively [37,38].

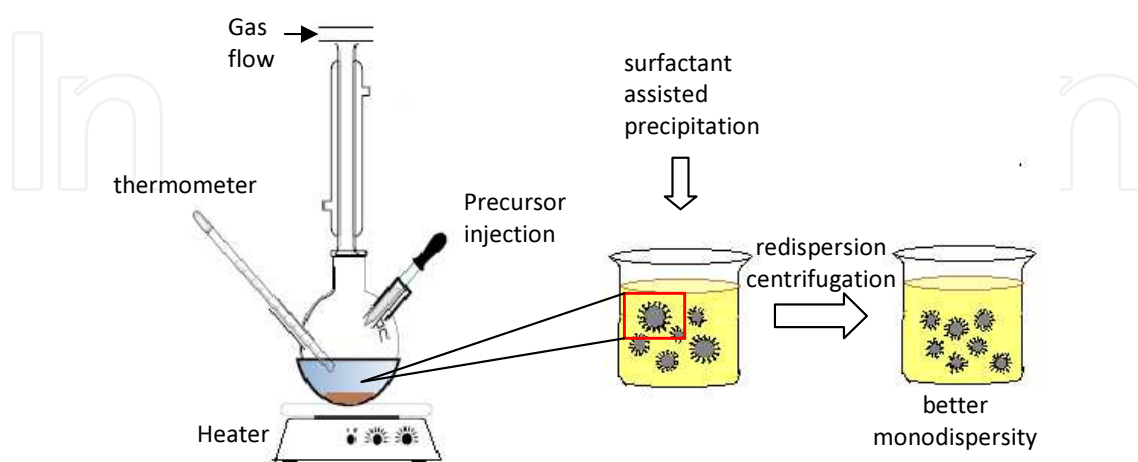
Ferrites	RT saturation magnetization $M_s$ (emu/g)	Anisotropy constant $K_1$ ( $\times 10^4$ J/m <sup>3</sup> )	Curie temperature $T_c$ (°C)	Superparamagnetic size $D_{sp}$ (nm)
$\text{Fe}_3\text{O}_4$	90-100	-1.2	585	25
$\text{NiFe}_2\text{O}_4$	56	-0.68	585	28
$\text{CoFe}_2\text{O}_4$	80-94	18-39	520	14
$\text{MnFe}_2\text{O}_4$	80	-0.25	300	25

**Table 1.** Some important magnetic parameters (room temperature saturation magnetization, first degree anisotropy constant, Curie temperature and superparamagnetic transition size) of ferrites [32]

Although MNPs can be prepared with several techniques including inert gas condensation [39], mechanical milling [40], spray pyrolysis [41], sol-gel [42], vapor deposition [43] and wet chemical processes [44], all the before mentioned key requirements for biomedical applications like crystallinity, size and shape uniformity together with other criterias for organic/inorganic coating of MNP, can be satisfied by hydrothermal chemical decomposition method, which is the most common one used in the synthesis of MNPs for biomedical applications. In this liquid

phase synthesis method, basically several organometallic precursors with suitable stoichiometric ratios are put in reaction in the presence of some organic surfactants or polymers. During the decomposition process at high temperature, in order to prohibit a possible oxidation, continuously an inert gas is fluxed through the mixture and at the end of the reaction the desired nanomaterial is obtained as a precipitate. The method enables the control on size of the MNPs through systematically change of the reaction parameters like concentration, reaction temperature and reaction time. In the method, MNPs with very narrow size distributions ( $\sigma \sim 10\%$ ) are synthesized and the distribution can be further improved (down to  $\sigma \sim 5\%$ ) by subsequently precipitating, redispersing and centrifuging of the particles, so-called as size selection processes (Figure.3). S. Sun and co-workers have published several pioneering papers in this area, where they have introduced hydrothermal routes for the synthesis of size-controlled MNPs for the first time and they have synthesized monodispersed iron oxide [45], other transition metal oxides [46] and iron-platinum [47] nanoparticles by using this method. The other advantages of the methods are the abilities of large quantity production of MNPs and the subsequent coating of them which will be explained in detail in the following of the chapter.

Another common technique used in MNP synthesis for biomedical applications is the coprecipitation method. It has some disadvantages against thermal decomposition method like lower crystallinity and lower monodispersity but the procedure is easier and at the end of the procedure larger amount of product can be yield. The method is based on the simultaneous nucleation and growth of magnetic cores by dissolving metal salt precursors in aqueous environment with changing pH and temperature [35]. Alternatively this reaction can be governed in a confined environment via microemulsion method. In this method the coprecipitation reaction takes place in some kind of “nano-reactors” called micelles, those are dispersions of two immiscible liquids like oil-in-water or water-in-oil (reverse micelle). The main advantage of the microemulsion is to ensure that the reaction occurs in an isolated media limiting the particle growth and it is possible to control the size of the magnetic core precisely by changing the size of the micelles [48].



**Figure 3.** An illustration of nanoparticle synthesis by high temperature thermal decomposition method and size selection processes.



### 3.2. Surface coating

In the design of MNPs, except the selection of a suitable magnetic core, fine tuning of surface coating materials represents a major challenge for the practical use of MNPs in clinical applications. The coating can consist of long-chain organic ligands or inorganic/organic polymers, where these ligands or polymers can be introduced during (in-situ coating) or after (post-synthetic coating) synthesis. During the in-situ coating, which is the procedure followed in co-precipitation synthesis technique, precursors of magnetic cores and coating materials are dissolved in the same reaction solution, and the nucleation of magnetic core and the coating occurs simultaneously. On the contrary in post-synthetic coating, which is the case in MNP synthesis with thermal decomposition technique, the surface coating materials are introduced after the formation of magnetic cores. In both procedures in order to link the surface molecules to magnetic cores, generally two different approaches; either end-grafting or surface-encapsulation are followed. In the former one, the coating molecules are anchored on magnetic core by the help of a single capping group at their one end, whereas in the latter generally polymers, already carrying multiple active groups, are attached on the surface of magnetic core with multiple connections resulting stronger and more stable coatings.

In some cases, surface modification of MNPs can dramatically change the magnetic properties hence the performance of the MNPs in biomedical applications depending on which coating material is used and how these materials are linked on the magnetic core surface as discussed above. Formation of chemical bonds between coating molecules and surface metal ions changes the surface spin structure and consequently the magnetic properties of coated MNPs with respect to uncoated ones. Actually it is difficult to discriminate between surface coating and finite size contributions, the latter being the effect of surface spin canting due to the minimization of magnetostatic energy at the surface and observed also in uncoated particles [49]. In finite size effect the canted spins at MNP's surface, do not respond to an external applied magnetic field as the bulk spins and give rise to a significant decrease in net magnetization, where this effect becomes dominant in smaller MNPs, since the volume fraction of disordered surface spins are increased [50]. On the other side, the physical origin of the surface coating effect on magnetic properties is still unclear and different results were reported in several studies for different kind of magnetic cores and coating materials. For example, Vestal and Zhang [51] have investigated the influence of the surface coordination chemistry on the magnetic properties of  $\text{MnFe}_2\text{O}_4$  nanoparticles by capping the 4, 12 and 25 nm sized  $\text{MnFe}_2\text{O}_4$  nanoparticles with a variety of substituted benzenes and substituted benzoic acid ligands and observed an increase in saturation magnetization, whereas a decrease is reported by Ngo et al. [52] for citrate coated 3 nm  $\text{CoFe}_2\text{O}_4$  nanoparticles. On the other hand, no significant change in the magnetic properties of 10 nm sized  $\gamma\text{-Fe}_2\text{O}_3$  nanoparticles is observed for different surface chemical treatments such as  $\text{NO}_3$ ,  $\text{ClO}_4$  and  $\text{SO}_4$  [53]. Another in-direct effect of the surface coating on magnetic properties, which should be mentioned, is to reduce the magnetic interparticle interactions through decreasing the distance between magnetic cores. This is important since strong interparticle interactions can alter the MNPs' overall magnetic properties i.e. superparamagnetic behaviour and diverse it from isolated ones.

Dormann and Fiorani have published several important papers investigating the magnetic interparticle interactions and the effect of coating [54-56].

The surface coating of MNPs plays a crucial role in biomedical applications by fulfilling more than one function at a time. The organic/inorganic surface coating is important for i) prohibiting agglomeration (clustering) of MNPs due to the above mentioned interparticle interactions and eventually providing the colloidal stability of water/organic solvent based suspensions/solutions (ferrofluids) prepared with MNPs ii) providing biocompatibility of MNPs by preventing any toxic ion leakage from magnetic core into the biological environment iii) serving as a base for further anchoring of functional groups such as biomarkers, antibodies, peptides etc. In the next paragraphs these functions of MNP's surface coating are discussed in detail.

### 3.2.1. *Stability*

The stabilization of the MNPs is crucial to obtain magnetic colloidal ferrofluids that are stable against aggregation both in a biological medium and in a magnetic field. By coating the MNPs, direct contacts among the particles are prevented by surface ligands and polymer chains extending into the medium. Therefore, no aggregates of particles will be formed or the rate of precipitation will be decreased depending on the degree of coverage of coating on the particles and the thickness of the coating layer. The stability of a magnetic colloidal suspension results from the equilibrium between attractive (magnetic dipole-dipole, van der Waals) and repulsive (electrostatic, steric) forces [57]. Controlling the strength of these forces is a key parameter to elaborate MNPs with good stability. A suitable surface coating should not only keep the MNPs apart from each other, eliminating the distance dependent attractive forces but should also ensure the charge neutrality and steric stability of MNPs in aqueous or organic media. The MNPs capped by hydrophilic surfactants like dodecylamine, sodium oleate can be easily dispersed in aqua but when they are stabilized by hydrophobic surfactants like oleic acid, oleylamine, which is the frequent case in the MNP synthesis with thermal decomposition method, they can only be dispersed in nonpolar solvents such as hexane, toluene or weak polar solvents such as chloroform. Therefore in order to stabilize these MNPs in aqueous media, different kind of polymers can be introduced on MNPs either by end-grafting or surface encapsulation or as an alternative strategy, the MNPs can be capped with amphiphilic molecules, consisting both hydrophobic and hydrophilic regions at their opposite ends via hydrophobic interactions resulting micelle-like structures [58].

### 3.2.2. *Toxicity (Biocompatibility)*

Another critical point which should be considered in the design of MNPs for biomedical applications is the toxicity of magnetic ion included in the magnetic core. The surface coating ensures a double-sided isolation both preventing the release of toxic ions from magnetic core into biological media and shielding the magnetic core from oxidation and corrosion. Among different types of MNPs iron oxide is by far the most commonly employed one for in vivo applications since iron is physiologically well tolerated. This is partially because the body is designed to process excess iron and it is already stored primarily in the core of the iron storage

protein called ferritin. Manganese (Mn) and Zinc (Zn) are other essential trace elements in human bodies, but their tolerable limit is much lower than iron's, so a surface manipulation is required [59]. Other elements, such as Co and Ni, which are desired for their high performance in hyperthermia application, are highly toxic and necessitates proper coatings when they are used in vivo. During in vivo applications once the MNPs are injected into the body, they are recognized by the body's major defence system (also known as reticuloendothelial system (RES)), which eliminates any foreign substance from blood stream. In this "opsonisation" process, MNPs are attacked by plasma proteins, which are sent by RES and are responsible for the clearance of MNPs. The specific surface coatings prevent the adsorption of these proteins, thus elongate the MNPs circulation time in blood and maximize the possibility to reach target tissues [60].

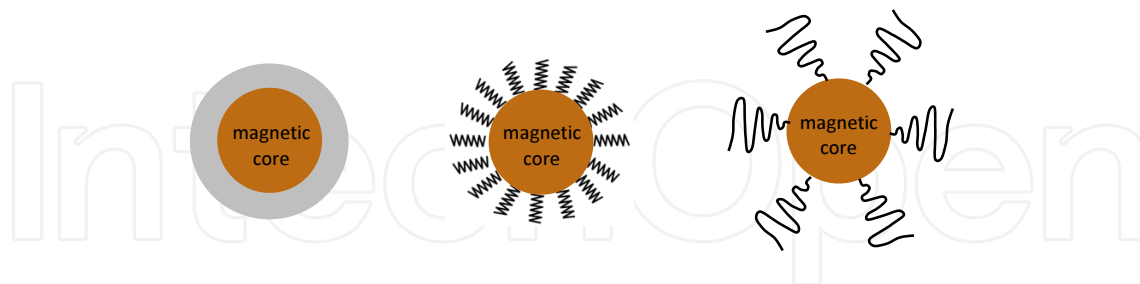
### 3.2.3. Functionalization

In order to enable the direct use of MNPs in biomedical applications, the MNPs should be further functionalized by conjugating them with functional groups. The surface coating provides suitable base for the attachment of these functional groups on MNPs. These groups such as antibodies, peptides, polysaccharides etc. permit specific recognition of cell types and target the nanoparticles to a specific tissue or cell type by binding to a cell surface receptor. Usually some linker molecules such as 1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide hydrochloride (EDCI), N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP), N-hydroxysuccinimide or N, N'-methylene bis acrylamide (MBA) are also used to attach the initial hydrophilic coated molecules to these targeting units [58]. In practice although the targeted cell population is recognized with high specificity, the fraction of targeted cells interacting with the antibody attached MNP is relatively low. Actually the effectiveness of biomedical applications depends more on cell-nanoparticle interactions than particle targeting. It is indicated that the cell membranes plays an important role in cell-nanoparticle interactions, either particle attachment to the cell membrane or particle uptake into the cell body. In order to clarify the nature of cell-nanoparticle interactions, usually phospholipid bilayers mimicking a cellular membrane or living cell membranes are used in studies.

### 3.2.4. Surface coating materials

For utilizing all the above mentioned functionalities of MNP's surface coating, different criteria of inorganic/organic molecules and polymers such as hydrophobic or hydrophilic, neutral or charged, synthetic or natural etc. are considered. The polymer coatings can be classified as natural polymers such as chitosan, dextran, rhamnose or synthetic polymers like polyethyleneglycol (PEG), polyvinyl alcohol (PVA), polyethyleneimine (PEI), polyvinylpyrrolidone (PVP). In some cases other organic molecules including oleic acid, oleylamine, dodecylamine and sodium oleate are also used to enhance water solubility of MNPs. On the other side, there are very less number of inorganic materials available for MNPs surface coatings, in which gold (Au) and silica (SiO<sub>2</sub>) are the most common used ones due to their biocompatibility. In Figure.4 a representative sketch of MNPs with different types of coatings are shown. In the next paragraphs, these coating materials are introduced in detail by summarizing their

outstanding properties and by discussing their feasibility in clinical applications with recent examples from literature.



**Figure 4.** A representative sketch of MNPs with different types of surface coatings: (a) inorganic materials (b) long chain organic molecules (c) organic polymers

#### 3.2.4.1. Inorganic surface coatings

##### Gold (Au)

Nanosized gold (Au) attracts too much attention due to its unique physical properties combined with chemical stability, biocompatibility and surface properties which permits its attachment to different chemical moieties. Surface modification of MNPs with biocompatible gold, both provides the in-water stabilization of particles by preventing their agglomeration and enables their functionalization by the attachment of several ligands on them. From applicative point of view, it also maintains the incorporation of some optical properties on MNPs promoting their use in dual-mode (optical and magnetic diagnosis) applications. Gold nanosurface shows surface plasmon resonance (SPR) phenomena, which triggers MNPs to strongly absorb and scatter near infrared wavelengths (Surface Enhanced Raman Scattering-SERS) accepted as “clear window” for deeper penetration of light into the human tissue [61]. In this respect gold modified MNPs, besides other applications, can simultaneously be used in SERS imaging, where detection in molecular level is possible enabling the diagnosis of diseases at very early stages. E. Umut et al. [12] have synthesized organically coated mono-dispersed gold-iron oxide hybrid nanoparticles following the chemical procedures introduced by W. Shi [62] and H. Yu [63], and showed that the superparamagnetic hybridnanoparticles have MRI contrast enhancement abilities associated with optical properties based on SPR phenomena, where the optical SPR absorbance depends on the geometry of synthesized hybrid nanoparticles. Similarly X. Ji et al. [64] have fabricated hybridnanoparticles where superparamagnetic iron oxide nanoparticles were embedded in silica and further coated with gold nanoshell, and they exhibit that the resulting nanoparticles both can improve the MRI contrast and have efficient photothermal effects when exposed to near infrared light. In another study H. Y. Park et al. [65] have synthesized  $\text{Fe}_3\text{O}_4\text{@Au}$  core-shell nanoparticles with controllable size ranging from 5 to 100 nm following a hetero-interparticle coalescence strategy and demonstrated the surface protein-binding properties and SERS effectiveness of synthesized nanoparticles. There are lots of similar studies in the literature, where gold nanoparticles or



nanosurfaces are introduced in different “smart” nanostructures for utilizing its optical properties together with surface binding properties in multifunctional manner [66-69].

### **Silica (SiO<sub>2</sub>)**

Another inorganic but “biofriendly” material used as MNPs’ coating is silica, which is known for its chemical stability and easy-to-formation. The first advantage of having surface enriched in silica is the presence of silanol groups, which can easily react with coupling agents providing strong attachment of surface ligands on MNPs [70]. As a second advantage, silica coating increases the stabilization of MNPs in liquid dispersions both by preventing the dipolar attractions and by increasing the surface charges hence the electrostatic repulsions between particles in non-aqueous dispersions. There are several successful methods available for the formation of silica coating, in which the mostly used one is the Stöber method, where a hydrolysis reaction of tetraethyl ortosilicate (TEOS) is governed in alcohol media under catalysis by ammonia [71]. In different studies reporting the synthesis of silica coated iron oxide nanoparticles, Y. H. Deng et al. have used Stöber method and obtained spherical coreshell nanoparticles by the condensation of TEOS in sol-gel form on pre-formed magnetite nanoparticles and have investigated the morphology and the thickness of the coating by systematically changing the used alcohol type or the amount of alcohol, ammonia and TEOS [72]. S. Santra et al. have applied water-in-oil microemulsion method again for the coating of previously synthesized iron oxide nanoparticles by using different nonionic surfactants and obtained as small as 1-2 nm and very uniformly sized (with standard deviation less than 10%) nanoparticles [73]. In another study D. K. Yi and colleagues, again by following a reverse microemulsion method, have synthesized homogenous silica-coated SiO<sub>2</sub>/Fe<sub>2</sub>O<sub>3</sub> nanoparticles with changing shell thickness at first stage and then they used these nanoparticles to derive mesoporous silica-coated SiO<sub>2</sub>/Fe<sub>2</sub>O<sub>3</sub> MNPs and hollow SiO<sub>2</sub> nanoballs [74]. In these studies the effect of coating and coating thickness on the magnetic properties of iron oxide were also investigated. As being in Au coated MNPs, attachment of different functional components to silica encapsulated MNPs either by embedding into or binding onto silica shell, smart engineering nanostructures realizing more than one function can be prepared [75,76].

#### *3.2.4.2. Organic surface coatings*

### **Chitosan**

Natural polymers and their derivatives have been widely utilized for coating of MNPs for in vivo applications. This is because such polymers are inexpensive and are known to be nonimmunogenic and nonantigenic in the body. They are usually anchored onto the surfaces of MNPs through carboxylate groups on their side chains. Chitosan is a natural polysaccharide cationic polymer, which is nontoxic, hydrophilic and biodegradable. There are lots of studies introducing chitosan as MNP carriers with the aim of use in biomedical applications. Y. Chang and D. Chen [77] have reported the preparation of 13,5 nm sized chitosan coated magnetite nanoparticles, where the chitosan was first carboxymethylated and then covalently bound on the surface of preformed magnetite nanoparticles via carbodiimide activation. They have also demonstrated that the synthesized nanoparticles can be efficiently used in magnetic ion separation. Thereafter instead of this two step



suspension crosslinking approach, J. Zhi et al. proposed an alternative method for in situ preparation of chitosan-magnetite nanocomposites in water-in-oil microemulsion [78]. By this method they have synthesized spherical chitosan nanoparticles in varying size from 10 nm to 80 nm with cubic shaped magnetite core. D. Kim et al. have prepared chitosan and -its derivative- starch coated iron oxide nanoparticles with the aim of treatment carcinoma cells by magnetic hyperthermia [79]. After performing in vitro cell cytotoxicity and affinity tests together with magneto-caloric measurements on magnetic fluids, they have shown that targeting of MNPs to cells was improved by using a chitosan coating and the coated MNPs are expected to be promising materials for use in magnetic hyperthermia. In another study published by Y. Ge et al. chitosan coated maghemite nanoparticles were modified with fluorescent dye by covalent bonding for dual-mode high efficient cellular imaging. They have shown that prepared nanoparticles could be efficiently internalized into cancer cells and serves as MRI contrast agents and optical probes for intravital fluorescence microscopy [80].

### **Dextran**

Another natural polymer is dextran, which is a neutral, branched polysaccharide composed of glucose subunits. Dextran is one of the most frequently chosen polymer because of its high biocompatibility. The studies conducted in order to find the biocompatibility of dextran have shown that the surface-immobilized dextran on MNPs is stable in most tissue environment, because dextran is resistant to enzymatic degradation [81]. However, the cellular uptake of dextran coated MNPs are not sufficient for most biomedical applications. One strategy to overcome this handicap has been reported by A. Jordan et al. as creating aminosilane groups on the surface of MNPs and by following this approach in vitro cellular uptake of such nanoparticles in carcinoma and glioblastoma cells was found to be thousand times higher than that of only dextran-coated MNPs [82]. With this method also much more efficient hyperthermia results have been obtained. Another pathway to increase the internalization of dextran coated particles by tumor cells is the further coupling of them with specific ligands. Some authors, with the aim of achieving better localized MRI contrast, have attached “transferrin” onto dextran coated MNPs and the cellular uptake of these ligand modified MNPs were two or four times higher compared to unmodified ones [83, 84]. There are many studies reporting different synthesis methods for preparing dextran-coated iron oxide nanoparticles [85,86] and investigating their interaction with cells [87,88].

### **Polyethylene Glycol (PEG)**

PEG is a neutral, hydrophilic, linear synthetic polymer that can be prepared with a wide range of terminal functional groups. By varying these functional groups, PEG can be binded to different surfaces. PEG coated MNPs reveal excellent stability and solubility in aqueous dispersions and in physiological media. Moreover the implementation of PEG as a surface coating of MNPs dramatically increases the blood circulation time of MNPs, enhancing their hinderence from the body's defence system. But as compared to multi-branched dextran coating, surface immobilized PEG permits limited grafting of further macromolecules since it has only one site available for ligand coupling. Related with the cellular uptake performance, Zhang et al. [89] have shown that the amount of internalized PEG coated MNPs into mouse

macrophage cells are much lower than uncoated MNPs. However, for breast cancer cells PEG modification of MNPs promotes better internalization of particles, where this situation is explained with the high solubility of PEG in physiological media hence the possibility of its diffusion into cell membranes [90]. In any case, as being in dextran coated MNPs, additional surface coupling of targeting ligands on PEG modified MNPs increases their cellular uptake. In order to attain a better intracellular hyperthermia efficacy, M. Suzuki et al. have attached monoclonal IgG antibody on the surface of PEG-modified magnetite nanoparticles and showed that cellular uptake of particles is improved [91].

Polymers / organic molecule	Properties	Advantages / Disadvantages
Chitosan	-natural, cationic, hydrophilic, linear, biodegradable	-can be used in non-viral gene delivery
Dextran	-natural, branched, hydrophilic, biocompatible	-permits the anchoring of biovectors and drugs when functionalized with amino groups
Polyethyleneglycol (PEG)	-synthetic, neutral, hydrophilic, linear, biocompatible	-remain stable at high ionic strengths of solutions with varying PH values, enhances blood circulation time (a few hours), permits functionalization
Polyethyleneimine (PEI)	-synthetic, cationic, linear or branched, non-biodegradable, toxic	-forms strong covalent bonds with MNP's surface, can be used for DNA and RNA delivery, but exhibit cytotoxicity
Polyvinylalcohol (PVA)	-synthetic, hydrophilic, biocompatible	- irreversibly binds on MNP's surface but can be used in temperature sensitive heating or drug release applications due to its decomposition temperatures (40-50 °C),
Polyvinylpyrrolidone (PVP)	-synthetic, branched, hydrophilic	-forms covalent bonds with drugs containing nucleophilic functional groups

**Table 2.** Some properties of different organic surface coating materials

### Polyvinyl Alcohol (PVA)

PVA is a hydrophilic, synthetic polymer. Coating of MNPs surface with PVA enhances the colloidal stability of ferrofluids prepared with these MNPs. But it has been suggested that PVA irreversibly binds on MNPs surface due to interconnected network with interface, which means a fraction of PVA remains associated with the nanoparticles despite repeated washing [92]. The residual PVA, in turn, influence different properties of nanoparticles such as particle size, zeta potential and surface hydrophobicity. Importantly, nanoparticles with higher amount of residual PVA had relatively lower cellular uptake. It is proposed that the lower intracellular uptake of nanoparticles with higher amount of residual PVA could be related to

the higher hydrophilicity of the nanoparticle surface [93]. A. P. Fink et al. have coated 9 nm sized iron oxide nanoparticles with unfunctionalized or carboxylate, amine or thiol functionalized PVA and observed that nanoparticles coated with PVA and carboxyl and thiol functionalized PVA were non-toxic to melanoma cells, whereas for the amine functionalized PVA nanoparticles, some cytotoxicity was observed particularly when the polymer concentrations were high [94].

### **Polyethyleneimine (PEI)**

PEI is a cationic, synthetic polymer and exist either as linear or branched forms. Although PEI is toxic and non-biodegradable, it has long been used for gene delivery thanks to its ability to bind with DNA [95]. Since it is a cationic polymer it can further interact with a wide variety of negatively charged complexes. Recently F. M. Kievit et al. have developed a complex MNP system, which is made of a superparamagnetic iron oxide nanoparticle (NP), which enables magnetic resonance imaging, coated with a novel copolymer comprised of short chain polyethylenimine (PEI) and poly(ethylene glycol) (PEG) grafted to the natural polysaccharide, chitosan (CP), which allows efficient loading and protection of the nucleic acids [96]. In this study they have illustrated the function of each component by comparative experiments and proposed that the designed complex MNP system is a potential candidate for safe in vivo delivery of DNA for gene therapy.

As should be summarized, there is a wide variety of coating materials could be attached on MNPs' surfaces by following different coating procedures and each of these materials have different advantages and disadvantages in biomedical applications depending on their characteristic properties like hydrophilicity, neutrality and structure. Table.2 lists some properties and outstanding advantages / disadvantages of different organic surface coating materials.

## **4. Conclusions**

In the last two decades, a lot of attention has been devoted to synthesis and characterization of functionalized iron or other transition metal oxide based MNPs, which have potential use in diagnosis and/or therapy in cancer treatment. These MNPs can act as contrast enhancement agents in diagnostic applications such as MRI and/or they can be used as carriers or locally heat releasers in therapeutic applications such as targeted drug delivery and magnetic hyperthermia, respectively. In the design of MNPs, the selection of the magnetic core and its surface modification with several organic/inorganic materials and polymers plays the major role effecting the performance of MNPs in these biomedical applications. Among different available magnetic ions, the correct selection of the magnetic core for MNPs requires careful and balanced consideration on material's properties such as chemical stability, toxicity and magnetization. The magnetic core further should be crystalline, small than a critical size and have a narrow size distribution where all these requirements together with proper in-situ or post synthetic surface coatings are satisfied by chemical methods like co-precipitation and thermal decomposition method. The surface coating is important for ensuring the biocompat-

ibility, colloidal stability and functionalization of MNPs, where a wide variety of coating materials are available like organic molecules/ polymers such as chitosan, dextran, Polyethyleneglycol (PEG), Polyethyleneimine (PEI), Polyvinylalcohol (PVA) or inorganic materials like silica and gold. Although much progress has been made on the fabrication of MNPs with delicate structure and enhanced surface properties, in using these MNPs for in vivo applications, major challenges still present like degradation, clearance of MNPs in the body, particle-cell interactions and changing physiological conditions like pH, temperature, blood pressure etc. which makes difficult to predict the behaviour of MNPs in biological medium.

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